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Novel antigens used to detect cell-mediated immune responses over time in *Mycobacterium avium* subsp. *paratuberculosis* infected cattle

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Background

Paratuberculosis is a chronic, granulomatous enteric infection in ruminants caused by *Mycobacterium* subsp. *avium paratuberculosis* (MAP). Early-stage MAP infection can be detected using diagnostics for cell mediated immune responses, e.g. the whole blood interferon gamma (IFN-γ) test. Available IFN-γ tests are using purified protein derivatives of MAP (PPDj) which are crude products consisting of undefined antigens with possible cross reactions toward other environmental bacteria.

Objective

The objective was to test novel recombinant antigens in the whole blood IFN-γ test to determine if some antigens could be excluded or combined in an optimized IFN-γ protocol .

Methods

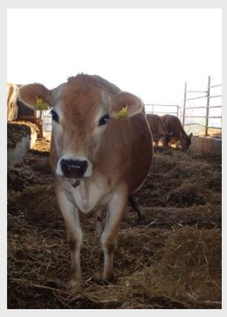
Fourteen novel antigens were selected in silico, expressed as recombinants in *Escherichia coli* and the purified products were used for testing.
Blood samples were collected 3 times with 4 and 5 week intervals from the same 30 heifers 15-24 months of age in a herd with known MAP infection.

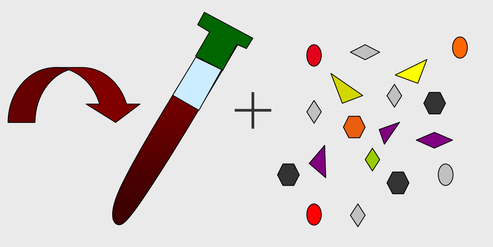
Novel MAP antigens tested in this study. Homologous sequences in *M. tuberculosis* and *M. bovis* genomes and gene names are listed.

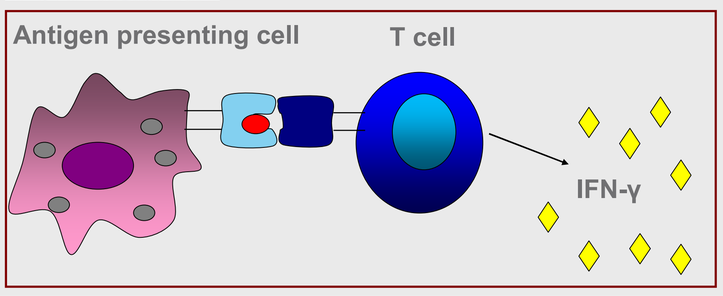
MAP antigen	*M.TB*	*M. bovis*	Gene	Characteristic
MAP0087	-	-	-	Not present in *M. avium* subsp. *avium*
MAP0160	Rv3891c	Mb3920c	*esxD*	ESAT-6 family member (M.TB and *M. bovis*)
MAP0217	Rv3803	Mb3833c	*fbpD*	Secreted proteins known to be expressed in M.TB
MAP1609	Rv1886c	Mb1918c	*fbpB*	Positive control (Ag85b)
MAP1662	Rv2301	Mb2323	*cut2*	Secreted proteins known to be expressed in M.TB
MAP2487c	Rv1284	Mb1315	*canA*	Latency proteins based on M.TB data
MAP2768c	Rv2659c	-	-	Latency proteins based on M.TB data
MAP2888	Rv2780	Mb2802/Mb2803	*ald*	Secreted proteins known to be expressed in M.TB
MAP3273c	Rv3131	Mb3155	-	Latency proteins based on M.TB data
MAP3701c	Rv0251c	Mb0257c	*hsp*	Latency proteins based on M.TB data
MAP3776	-	-	-	Not present in *M. avium* subsp. *avium*
MAP3783	Rv0287	Mb0295	*esxG*	Immunological hot spot region
esxH	Rv0288	Mb0296	*esxH*	ESAT-6 family member (M.TB and *M. bovis*)
esxK	Rv1197	Mb1229	*esxK*	ESAT-6 family member (M.TB and *M. bovis*)
esxU	Rv3445	Mb3475	*escU*	ESAT-6 family member (M.TB and *M. bovis*)

IFN-γ test protocol:

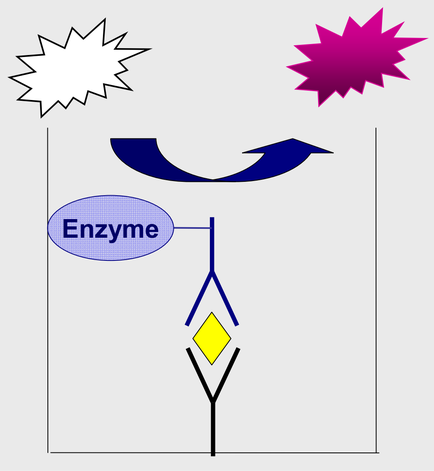
1) Cultivation of whole blood with MAP antigens



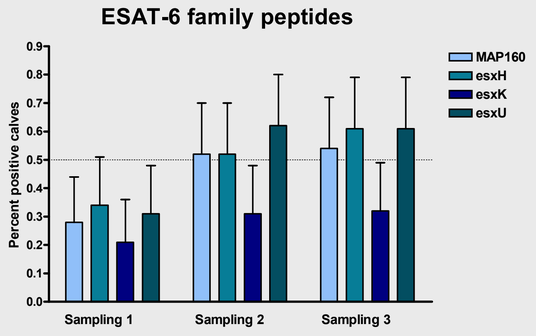


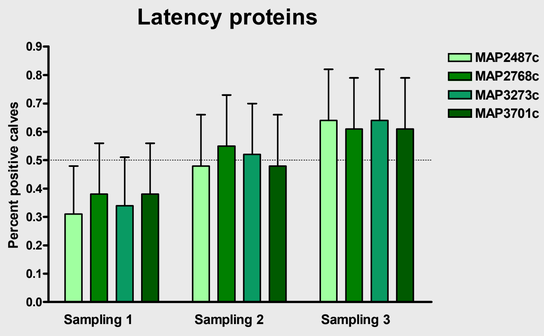


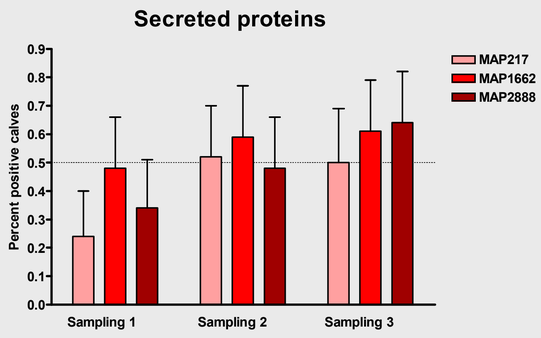
2) IFN- γ detection by ELISA

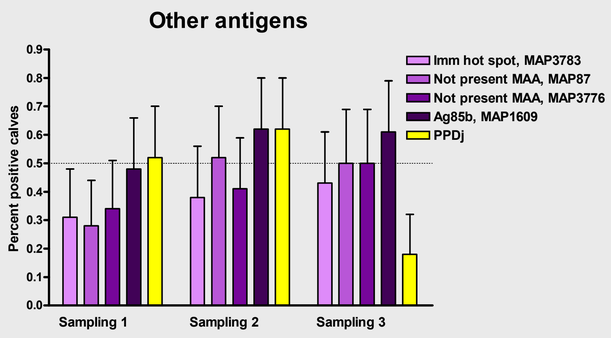


Results









Interpretation

Cut-offs used to discriminate test-positives and test-negatives for each antigen was based on samples from 60 heifers 15-24 months of age from a non-infected herd.
Samples were excluded if the IFN-γ response to SEB <1500 pg/ml or if IFN-γ response to PBS > 250 pg/ml.

Conclusions

- PPDj stimulations tested 50% and 60% of the animals as test positive at the two first samplings, and less than 20% at the third sampling.
- Ag85b (MAP1609) tested 50% to 60% of the animals positive at all three samplings.
- The groups of latency proteins and secreted proteins tested 50% to 60%of the animals positive at the last two samplings.
- ESAT-6 family peptides (MAP160, exsH and esxU) tested 50% to 60% of the animals as positive at the last two samplings and could be promising diagnostic candidates.

Future work

Antigens with high MAP specificity will be selected for inclusion in the IFN-γ test. The best combination of antigens remains to be investigated.

Acknowledgements

Technicians Abdellatif El Ghazi and Sardar Ahmad have done most of the ELISAs. This study was co-funded by the European Commission within the Sitxh Framework Programme, as part of the project ParaTBTools (contract no. 023106 (FOOD)).